

52. The method of claim 15 wherein reactivation of the patient's thymus restores the patient's peripheral T cell levels to a level corresponding to that found in a pre-pubertal person.

53. The method of claim 15 resulting in a vaccine response by the patient's immune system that is comparable to the response of a pre-pubertal patient.

54. A kit for use in improving a patient's immune response comprising the following components:

an LHRH analog;

at least one compound selected from the group consisting of interleukin 7, stem cell factor, interleukin 2, interleukin 15, granulocyte colony stimulating factor, keratinocyte growth factor, steroid receptor modulator, enhancing compound, immunosuppressant, suppressor of adrenal gland production of sex steroids; and a carrier. *AC*

#### IN THE SPECIFICATION:

*AC* On page 16, paragraph 0068, line 7, after "sex steroid analog," and before "preferably" please insert --or--.

On page 16, paragraph 0068, line 10 after "the LHRH receptor (LHRH-R):" and before "Buserelin" please insert --Eulexin,--.

On page 17, paragraph 0070, line 2 please delete "once" and insert therefore --one -- three times--.

On page 35, paragraph 0157, line 17, after "3 month" and before "depot" please insert therefore --(or 3 times one month)--.

#### IN THE DESCRIPTION OF FIGURES:

Beginning on page 6, paragraph 0023 through page 8, paragraph 0031, line 16, please amend the description for figures 1 through 9 to read as follows:

**[0023]** Figure 1 A and B: Changes in thymocyte number pre- and post-castration. Thymus atrophy results in a significant decrease in thymocyte numbers with age. Aged (2-year old) mice were surgically castrated and analysed for (A) thymus weight in relation to body weight and (B) total cells per thymus, at 2-4 weeks post-castration. A significant decrease in thymus weight and cellularity was seen with age compared to young adult (2-month) mice. This was restored by castration. At 3-weeks post-castration thymic hypertrophy was observed and was returned to young adult levels by 4-weeks post-castration. Results are expressed as mean  $\pm$ 1SD of 4-8 mice per group. \*\* =  $p \leq 0.01$ ; \*\*\* =  $p \leq 0.001$  compared to young adult and post-castration mice.

**[0024]** Figure 2 A-C: Aged (2-year old) mice were surgically castrated and analysed at 2 and 4 weeks post-castration for peripheral lymphocyte populations. (A) Total lymphocyte numbers in the spleen. Spleen numbers remain constant with age and post-castration. (B) The ratio of B cells to T cells did not change with age or post-castration, however (C) a significant decrease in the CD4<sup>+</sup>:CD8<sup>+</sup> T cell ratio was seen with age. This was restored by 4-weeks post-castration. Data is expressed as mean $\pm$ 1SD of 4-8 mice per group. \*\*\* =  $p \leq 0.001$  compared to young adult (2-month) and 4-week post-castrate mice.

**[0025]** Figure 3: Fluorescence Activated Cell Sorter (FACS) profiles of CD4 vs. CD8 thymocyte populations with age and post-castration. Aged (2-year old) mice were castrated and the thymocyte subsets analysed based on the markers CD4 and CD8. Representative FACS profiles of CD4/CD8 dot plots are shown for CD4<sup>+</sup>CD8<sup>-</sup>DN, CD4<sup>+</sup>CD8<sup>+</sup>DP, CD4<sup>+</sup>CD8<sup>-</sup> and CD4<sup>+</sup>CD8<sup>+</sup> SP thymocytes. No difference was seen in the proportions of any CD4/CD8 defined subset with age or post-castration.

**[0026]** Figure 4: Aged (2-year old) mice were castrated and injected with a pulse of bromodeoxyuridine (BrdU) to determine levels of proliferation. Representative histogram profiles of the proportion of BrdU<sup>+</sup> cells within the thymus with age and post-castration are shown. No difference in the proportion of proliferating cells within the total thymus was observed with age or post-castration.

**[0027]** Figure 5 A-D: Effects of age and castration on proliferation of thymocyte subsets. (A) Proportion of each subset that constitutes the total proliferating population—The proportion of CD8<sup>+</sup> T cells within the proliferating population is significantly increased. (B) However, a significant decrease in the proportion of DN (CD4-CD8-) thymocytes proliferating was seen with age. Post-castration, this was restored and a significant increase in proliferation within the CD4<sup>+</sup>CD8<sup>+</sup> SP thymocytes was observed. (C) No change in the total proportion of BrdU<sup>+</sup> cells within the TN subset was seen with age or post-castration. However (D) the significant decrease in proliferation of the TN1 (CD44<sup>+</sup>CD25<sup>-</sup>) subpopulation with age is not returned to normal levels by 4 weeks post-castration. Results are expressed as mean±1SD of 4-8 mice per group. \* =  $p \leq 0.05$ ; \*\*\* =  $p \leq 0.001$  compared to young adult (2-month) mice.

**[0028]** Figure 6 A-C: Aged (2-year old) mice were castrated and were injected intrathymically with FITC to determine thymic export rates. The number of FITC<sup>+</sup> cells in the periphery were calculated 24 hours later. (A) A significant decrease in recent thymic emigrant (RTE) cell numbers was observed with age. Following castration, these values had significantly increased by 2 weeks post-cx. (B) The rate of emigration (export/total thymus cellularity) remained constant with age but was significantly reduced at 2 weeks post-cx. (C) With age, a significant increase in the ratio of CD4<sup>+</sup> to CD8<sup>+</sup> RTE was seen and this was normalised by 1-week post-cx. Results are expressed as mean±1SD of 4-8 mice per group. \*\* =  $p \leq 0.01$ ; \*\*\* =  $p \leq 0.001$  compared to young adult mice. ^ =  $p \leq 0.001$  compared to castrated mice.

**[0029]** Figure 7 A-C: Changes in thymus (A), spleen (B) and lymph node (C) cell numbers following treatment with cyclophosphamide, a chemotherapy agent. Young (3-month old) mice were depleted of lymphocytes using cyclophosphamide. Mice were either sham-castrated or castrated on the same day as cyclophosphamide treatment. (A) A significant increase in thymus cell number was observed in castrated mice compared to sham-castrated mice. (B) Castrated mice also showed a significant increase in spleen cell number at 1-week post-cyclophosphamide treatment. (C) A significant increase in lymph node cellularity was also observed with castrated mice at

1-week post-treatment. Results are expressed as mean $\pm$ 1SD of 4-8 mice per group. \*\*\* =  $p \leq 0.001$  compared to castrated mice.

**[0030]** Figure 8 A-C: Changes in thymus (A), spleen (B) and lymph node (C) cell numbers following irradiation and castration on the same day. Note the rapid expansion of the thymus in castrated animals when compared to the non-castrate group at 2 weeks post-treatment. No difference in spleen (B) or lymph node (C) cell numbers was seen with castrated mice. Lymph node cell numbers were still chronically low at 2-weeks post-treatment compared to control mice. Results are expressed as mean $\pm$ 1SD of 4-8 mice per group. \* =  $p \leq 0.05$  compared to control mice; \*\*\* =  $p \leq 0.001$  compared to control and castrated mice.

*A3*  
**[0031]** Figure 9 A-C: Changes in thymus (A), spleen (B) and lymph node (C) cell numbers following irradiation (625 Rads) one week after surgical castration. A significant increase in thymus regeneration was observed with castration (A). No difference in spleen (B) or lymph node (C) cell numbers was seen with castrated mice. Lymph node cell numbers were still chronically low at 2-weeks post-treatment compared to control mice. Results are expressed as mean  $\pm$  1SD of 4-8 mice per group. + =  $p \leq 0.05$ ; \*\* =  $p \leq 0.01$  compared to control mice; \*\*\* =  $p \leq 0.001$  compared to control and castrated mice.

Beginning on page 8, paragraph 0033, line 15, please amend the descriptions for Figures 11 through 15 to read as follows:

*A4*  
**[0033]** Figure 11 A and B: Lymph node cellularity following foot-pad immunization with Herpes Simplex Virus-1 (HSV-1). Note the increased cellularity in the aged post-castration as compared to the aged non-castrated group (A). Bottom graph illustrates the overall activated cell number as gated on CD25 vs. CD8 cells by FACS (B).

**[0034]** Figure 12 A-C: V $\beta$ 10 expression on CTL (cytotoxic T lymphocytes) in activated LN (lymph nodes) following HSV-1 inoculation. Despite the normal V $\beta$ 10 responsiveness in aged mice overall, in some mice a complete loss of V $\beta$ 10 expression was observed. Representative histogram profiles are shown. Note the diminution of a

clonal response in aged mice and the reinstatement of the expected response post-castration.

**[0035]** Figure 13 A-C: Castration restores responsiveness to HSV-1 immunization. (A) Aged mice showed a significant reduction in total lymph node cellularity post-infection when compared to both the young and post-castrate mice. (B) Representative FACS profiles of activated ( $CD8^+CD25^+$ ) cells in the LN of HSV-1 infected mice. No difference was seen in proportions of activated CTL with age or post-castration. (C) The decreased cellularity within the lymph nodes of aged mice was reflected by a significant decrease in activated CTL numbers. Castration of the aged mice restored the immune response to HSV-1 with CTL numbers equivalent to young mice. Results are expressed as mean  $\pm 1$  SD of 8-12 mice. \*\* =  $p \leq 0.01$  compared to both young (2-month) and non-castrated mice.

*Ag*  
**[0036]** Figure 14: Popliteal lymph nodes were removed from mice immunized with HSV-1 and cultured for 3 days. CTL assays were performed with non-immunized mice as control for background levels of lysis (as determined by  $^{51}Cr$ -release). Results are expressed as mean of 8 mice, in triplicate  $\pm 1$ SD. Aged mice showed a significant ( $p \leq 0.01$ , \*) reduction in CTL activity at an E:T ratio of both 10:1 and 3:1 indicating a reduction in the percentage of specific CTL present within the lymph nodes. Castration of aged mice restored the CTL response to young adult levels. \* =  $p \leq 0.01$  compared to young adult and post-castrate aged mice.

**[0037]** Figure 15 A and B: Analysis of  $CD4^+$  T cell help and  $V\beta$  TCR response to HSV-1 infection. Popliteal lymph nodes were removed on D5 post-HSV-1 infection and analysed ex-vivo for the expression of (a) CD25, CD8 and specific TCR $V\beta$  markers and (b)  $CD4/CD8$  T cells. (A) The percentage of activated ( $CD25^+$ )  $CD8^+$  T cells expressing either  $V\beta 10$  or  $V\beta 8.1$  is shown as mean  $\pm 1$ SD for 8 mice per group. No difference was observed with age or post-castration. (B) A decrease in  $CD4/CD8$  ratio in the resting LN population was seen with age. This was restored post-castration. Results are expressed as mean  $\pm 1$ SD of 8 mice per group. \*\*\* =  $p \leq 0.001$  compared to young and castrate mice.

Beginning on page 10, paragraph 0041, line 17 through line 25 please amend the description for Figure 19 to read as follows:

AS **[0041]** Figure 19 A and B: Myeloid and lymphoid dendritic cell (DC) number after lethal irradiation, fetal liver reconstitution and castration. (n= 3-4 mice for each test group.) Control (white) bars on the following graphs are based on the normal number of dendritic cells found in untreated age matched mice. (A) Donor-derived myeloid dendritic cells—Two weeks after reconstitution DC were present at normal levels in noncastrated mice. There were significantly more DC in castrated mice at the same time point. (\*p≤ 0.05). At four weeks DC number remained above control levels in castrated mice. (B) Donor-derived lymphoid dendritic cells—Two weeks after reconstitution DC numbers in castrated mice were double those of noncastrated mice. Four weeks after treatment DC numbers remained above control levels.

Beginning at page 11, paragraph 0043, line 5 through page 12, line 3, please amend Figures 21 through 23 to read as follows:

AS **[0043]** Figure 21 A-C: Changes in T cells and myeloid and lymphoid derived dendritic cells (DC) in bone marrow of castrated and noncastrated mice after fetal liver reconstitution. (n=3-4 mice for each test group.) Control (white) bars on the following graphs are based on the normal number of T cells and dendritic cells found in untreated age matched mice. (A) T cell number—Numbers were reduced two and four weeks after reconstitution in both castrated and noncastrated mice. (B) Donor derived myeloid dendritic cells—Two weeks after reconstitution DC cell numbers were normal in both castrated and noncastrated mice. At this time point there was no significant difference between numbers in castrated and noncastrated mice. (C) Donor-derived lymphoid dendritic cells—Numbers were at normal levels two and four weeks after reconstitution. At two weeks there was no significant difference between numbers in castrated and noncastrated mice.

**[0044]** Figure 22 A and B: Change in total and donor (CD45.2<sup>+</sup>) lymph node cell numbers in castrated and noncastrated mice after fetal liver reconstitution. (n=3-4 mice